Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/006822

International filing date: 01 March 2005 (01.03.2005)

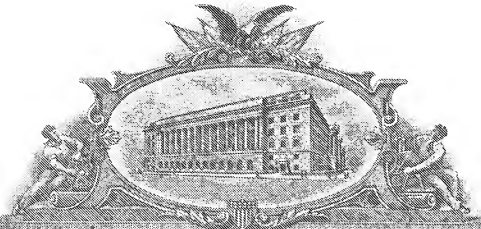
Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/551396
Filing date: 09 March 2004 (09.03.2004)

Date of receipt at the International Bureau: 25 April 2005 (25.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 18, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: ***60/551,396***
FILING DATE: ***March 09, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/06822***

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (08-03)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EJ624593993US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Kai W.	Wucherpfennig	Brookline, MA
James	Rasmussen	Cambridge, MA
Bei	Yu	West Roxbury, MA

Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

METHODS AND COMPOSITION FOR TREATMENT OF AUTOIMMUNE DISEASES

CORRESPONDENCE ADDRESS

Direct all correspondence to:

[x] Customer Number: 28120

OR

[ ] Firm or Individual Name: Ropes & Gray LLP

Address: Patent Group, One International Place

City: Boston | State: MA | Zip: 02110

Country: US | Telephone: 617-951-7000 | Fax: 617-951-7050

ENCLOSED APPLICATION PARTS (*check all that apply*)

[x] Specification *Number of Pages* 40 [ ] CD(s), Number

[ ] Drawing(s) *Number of Sheets* [ ] Other (specify):

[ ] Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[ ] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check or money order is enclosed to cover the filing fees.

[x] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 18-1945

[ ] Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): 160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[x] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE

TYPED OR PRINTED NAME: Erika Takeuchi

TELEPHONE: (212) 497-3625

Date: March 9, 2004

REGISTRATION NO. *(if appropriate)*: 55,661

Docket Number: PEPT-P60-006

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EJ624593993US, in an envelope addressed to: MS Provisional Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 9, 2004 Signature: Linda Blake (Linda Blake)

PTO/SB/17 (10-03)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 160.00

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	March 9, 2004
First Named Inventor	Kai W. Wucherpfennig
Examiner Name	Not Yet Assigned
Art Unit	N/A
Attorney Docket No.	PEPT-P60-006

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit Card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 18-1945

Deposit Account Name: Ropes & Gray LLP

The Director is authorized to: *(check all that apply)*

☒ Charge fee(s) indicated below ☒ Credit any overpayments

☒ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	160.00

SUBTOTAL (1) ($) 160.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims	Fee from below	Fee Paid
Total Claims		-** =		x	=
Independent Claims		-** =		x	=
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0.00

***or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge – late filing fee or oath	
1052	50	2052	25	Surcharge – late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unevoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
Other fee (specify)					

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** ($) 0.00

SUBMITTED BY				(Complete *if applicable*)	
Name (Print/Type)	Erika Takeuchi	*Registration No. (Attorney/Agent)*	55,661	*Telephone*	(212) 497-3625
Signature				*Date*	March 9, 2004

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EJ624593993US, in an envelope addressed to: MS Provisional Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 9, 2004 Signature: _____ (Linda Blake)

9390265_1.DOC

Application No. (if known): Not Yet Assigned | Attorney Docket No.: PEPT-P60-006

Certificate of Express Mailing Under 37 CFR 1.10

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail, Airbill No. EJ624593993US in an envelope addressed to:

MS Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on March 9, 2004
Date

Linda Blake
Signature

Linda Blake
Typed or printed name of person signing Certificate

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

Application (40 pages)

Express Mail Label No. EJ624593993US

Title:

METHODS AND COMPOSITION FOR TREATMENT OF AUTOIMMUNE DISEASES

Inventors:

Kai W. Wucherpfennig

James Rasmussen

Bei Yu

METHODS AND COMPOSITION FOR TREATMENT OF AUTOIMMUNE DISEASES

BACKGROUND OF THE INVENTION

[0001] Certain human leukocyte antigen (HLA) alleles occur in a higher frequency in individuals with particular diseases than in the general population. HLA locus encodes major histocompatibility complex (MHC) genes of human. MHC molecules exist in two forms, class I and class II, both encoded within a single gene complex. MHC genes are highly polymorphic: some loci have up to about several hundred alleles in the human population (Hansen, T.H. *et al.* 1993 In "Fundamental Immunology" Ed. Paul, W.E., Raven Press, New York, NY, p.577).

[0002] Class I MHC molecules are 45 kD transmembrane glycoproteins, noncovalently associated with another glycoprotein, the 12 kD β-2 microglobulin. The latter is not inserted into the cell membrane, and is encoded outside the MHC region of the genome. Human class I molecules are of three different isotypes, termed HLA-A, -B, and -C, encoded in separate loci. The tissue expression of class I molecules is ubiquitous and codominant. The three-dimensional structure of several human and murine class I molecules have been resolved (Bjorkman, P.J. *et al.* (1987) *Nature* 329: 506; Garrett, T.P.J. *et al.* (1989) *Nature* 342: 692; Madden, D.R. *et al.* (1991) *Nature* 353: 321; Fremont, D.H. *et al.* (1992) *Science* 257: 919). The three class I isotypes, as well as their allelic forms, have different peptide binding specificities, depending on polymorphic residues within the binding site (Falk, K. *et al.* (1991) *Nature* 351: 290; Falk, K. *et al.* (1992) *Eur. J. Immunol.*, 22: 277).

[0003] Class II MHC molecules are noncovalently associated heterodimers of two transmembrane glycoproteins, the 35 kD α chain and the 28 kD β chain. In humans, class II molecules occur as three different isotypes, termed HLA-DP, -DQ, and -DR. There are a minimum of six α and eight β genes, which are arranged in distinct clusters. Polymorphism in DR is restricted to the β chain, whereas both chains are polymorphic in the DP and DQ isotypes. Structural variation in class II gene products is linked to functional features of immune recognition, leading to individual variations in histocompatibility, immune recognition, and susceptibility to disease. The two types of dimers along the HLA cell-surface HLA-DR class II molecules are made up of DRα polypeptide associated with $DR\beta_1$, $DR\beta_2$, $DR\beta_3$ or $DR\beta_4$

polypeptide. The two types of structural variations comprise primary amino acid sequences which differ by as much as 35%. The class II polypeptide chain possesses domains that are specific structural subunits containing variable sequences that distinguish among class II α genes and class II β genes. These allelic variation sites have been suggested to form antigen binding clefts, which represent individual structural differences in immune recognition. Class II molecules are expressed codominantly, but in contrast to class I, exhibit a restricted tissue distribution: they are present only on the surface of cells of the immune system. Such cells include antigen-presenting cells, for example, macrophages, dendritic cells, and Langerhans cells; epithelial tissue cells that interact with immune system, including thymic epithelial cells; B lymphocytes, monocytes and mast cells; and T cells when they are induced.

[0004] The three-dimensional structure of three different DR molecules and a DQ molecule of class II MHC has been determined (Brown, J.H. *et al.* (1993), *Nature* 364: 33; Stern, L.J. *et al.* (1994) *Nature* 388: 215; Ghosh, P. *et al.* (1995) *Nature* 378: 457; Dessen, A. *et al.* (1997) *Immunity*, 7: 473; Lee, K. *et al.* (2001) *Nature Immunol.* 2(6): 501-507). Overall, their structure is very similar to that of class I molecules. The peptide binding site is composed of the first domains of α and β chain, which, in contrast to class I, is open on both sides, allowing the binding of longer (12-24 residues long) peptides (Chicz, R.M. *et al.* (1992) *Nature*, 358: 764). An additional binding site on the second domain of both α and β chains interacts with the CD4 molecule, expressed selectively on helper T (Th) cells. This molecule has a co-receptor function for T helper (Th) cells, analogous to that of CD8 for cytotoxic T (Tc) cells.

[0005] A peptide bound to a class II MHC molecule is presented in such a way that particular T cells are activated. There are generally two types of T cells: T helper 1 (Th1) and T helper 2 (Th2). Th1 cells participate in providing cell-mediated immunity, which is generally pro-inflammatory. When activated, Th1 cells produce pro-inflammatory cytokines such as interferon(IFN)-γ and interleukin (IL)-2. Th2 cells participate in providing humoral immunity, which is generally non-inflammatory. When activated, Th2 cells produce non-inflammatory cytokines such as IL-4, IL-5 and IL-9. The activated T cells may also be induced to proliferate or to undergo apoptosis. Thus, peptides bound to, and presented by, MHC molecules may activate either Th1 or Th2, shifting the balance of pro-inflammatory and non-inflammatory responses, depending on the identity of the peptides.

[0006] A large body of evidence has demonstrated that susceptibility to many diseases, in particular autoimmune diseases, is strongly associated with specific alleles of the major histocompatibility complex (reviewed in Tiwari, J., and Terasaki, P. (1985), "HLA and disease association," New York; Springer Verlag). Although some class I-associated diseases exist, most autoimmune conditions have been found to be associated with class II alleles. MHC class II molecules are of great importance in the selection and activation of CD4+ T lymphocytes, which regulate the immune responses against protein antigens. Genomic analysis has identified specific individual allelic variants of HLA in associations with Hodgkin's disease, multiple sclerosis, rheumatoid arthritis, pemphigus vulgaris, Type I diabetes mellitus, and celiac disease, among others. (Thomson, G. (1995) *Crit. Rev. Clin. Lab. Sci.* 32:183-219; Nepom, G.T. and Erlich, H. (1991) *Annu. Rev. Immunol.* 9:493-525; Tiwari, J.L., above)

[0007] Type 1 diabetes (*i.e.*, Insulin-dependent diabetes mellitus, (IDDM)) represents 20% of all human diabetes, and is the most serious form of the disease, with highest morbidity and mortality. Up to 800,000 people in the U.S. are estimated to have IDDM, with about 30,000 new cases diagnosed each year. The incidence of IDDM has been rising over the past few decades in certain regions of the US and some European countries, particularly in Finland and England. Some complications arising from long-standing diabetes are vascular disease, microvascular disease, eye complications, diabetic nephropathy, diabetic neuropathy, diabetic foot problems, and skin and mucous membrane problems.

[0008] IDDM is a progressive autoimmune disease, in which the β cells of the pancreas that produce insulin are slowly destroyed by the body's own immune system. Certain proteins, such as glutamic acid decarboxylase (GAD), insulin, and islet cell antigens, serve as autoantigens, becoming targets of self-attack of the immune system. It is unknown what triggers this cascade of aberrant immune events, but IDDM susceptibility and resistance has been associated with the alleles of HLA-DQB1 and DQA1 loci. Such HLA-DQ molecules are the combined protein products of specific HLA-DQB1 and DQA1 alleles known as DQB1*0201, DQB1*0302, DQB1*0304, DQB1*0401, DQB1*0501, DQB1*0502; and DQA1*0301, DQA1*0302, DQA1*0303, DQA1*0501. These alleles may be encoded on one haplotype ("cis" alleles) such as DQB1*0201-DQA1*0501-DRB1*0301 and DQB1*0302-DQA1*0301-DRB1*0401. Alternatively, the alleles may be encoded on different haplotypes ("trans" alleles). An example of "trans" alleles is the combination of DQB1*0201 on DQB1*0201-DQA1*0501-

DRB1*0301 and DQA1*0301 on DQB1*0301-DQA1*0301-DRB1*0404. Individuals carrying both DQB1*0201-DQA1*0501 and DQB1*0302-DQA1*03 haplotypes have the highest risk of developing IDDM. (Yu *et al.* (2000) *Eur. J. Immunol.* 30:2497-2506). There is a strong correlation between Additionally, 95% of Caucasians with IDDM carry the alleles DRB1*0301 or DRB1*0401, or both.

[0009] Currently, treatment of IDDM requires chronic administration of insulin to control hyperglycemia. Uncontrolled hyperglycemia can further damage the insulin-producing pancreatic β cells, and in the long term, create greater insulin deficiencies. Currently, oral sulfonylureas and insulin injections are the only two therapeutic agents available in the United States for treatment of IDDM. Both agents have the potential for inducing hypoglycemia as a side effect, reducing the blood glucose concentration to dangerous levels. There is no generally applicable and consistently effective means of maintaining an essentially normal fluctuation in glucose levels in IDDM. The resultant treatment attempts to minimize the risks of hypoglycemia while keeping the glucose levels below a target value. The drug regimen is combined with dietary intake of carbohydrates to keep glucose levels in control. However, to date, there is no cure for IDDM.

[0010] Celiac disease, also known as celiac sprue or gluten-sensitive enteropathy, is a disease that results from defective gastrointestinal absorption due to hypersensitivity to cereal grain storage proteins, including glutens or its product gliadin and glutenin, present in wheat, barley, and oats. The disease is caused by CD4 T cells that recognize gliadin as dietary antigen and these cells induce a Th1-mediated chronic inflammatory response. Symptoms include diarrhea, weight loss, and steatorrhea, villous atrophy and malabsorption are seen. It may also be associated with dermatitis herpetiforms, a vesicular skin eruption. Celiac disease is associated with alleles DQB1*0302 and DQB1*0201 combined with DQA1*0301 and DQA1*0501. 95% of patients carry either DQB1*0201 or DQB1*0302. The strong HLA association is believed to be due to the capacity of DQ molecules encoded by DQB1*0201, DQA1*0501, DQB1*0302 and DQA1*0301 to efficiently present deaminated variants of glutamine-rich peptides derived from gliadin and glutenin.

[0011] Of the two classes of MHC molecules, class II is the primary target for immunosuppressive intervention for the following reasons: First, MHC-II molecules activate T helper (Th) cells that are central to immunoregulation, and are responsible for most of the

immunopathology in inflammatory diseases. Second, most autoimmune diseases are genetically associated with class II alleles. Third, MHC-II molecules are expressed selectively on cells of the immune system, whereas MHC-I are present on most somatic cells.

[0012] A pharmaceutical agent targeting class II MHC molecules offers several advantages over most available immunosuppressive drugs. First, it would represent a disease mechanism-based intervention, which is expected to interrupt the initial event in the pathogenic cascade. Second, it can be designed to be selective for only a few class II allotypes, leaving the remainder of the antigen presenting system available for protective responses against pathogens, and therefore causing fewer immunocompromising side effects than most immunosuppressive drugs. Third, the methods and compounds could be applied without any specific knowledge of the actual autoantigens causing the disease.

[0013] To date, methods and compositions targeting the HLA-DR subclass molecules have been described, but not those that target the HLA-DQ subclass molecules.

SUMMARY OF THE INVENTION

[0014] The present invention provides methods and compositions for treating autoimmune diseases and other unwanted immune reactions comprising administering a copolymer that binds to one or more HLA-DQ molecules and modulates DQ-restricted T cell responses. In certain preferred embodiments, the copolymers of the invention bind to HLA-DQA1 molecules, and in even more preferably to one or more of HLA molecules encoded in the alleles DQA1*0501-DQB1*0201, DQA1*0301, DQB1*0401, and DQA1*03-DQB1*0302. Exemplary disorders that can be treated using the subject DQ-directed copolymers include insulin-dependent diabetes mellitus (IDDM); celiac disease; rheumatoid arthritis; steroid sensitive nephrotic syndrome; mesengial IgA nephropathy; narcolepsy; neurological multiple sclerosis; relapsive polychondritis ; dermatological disorders such as dermatitis herpetiformis, atopic dermatitis, Behcet's disease, pemphigus, psoriasis; primary Sjögren's syndrome; systemic vasculitides; erythematosus; gastrointestinal disorders such as Crohn's disease; respiratory disorders such as Sommer type hypersensitivity pneumonitis, and autoimmune thyroid disease (AITD). In even more preferred embodiments, the copolymers of the present invention bind to certain HLA-DQ molecules that predispose the carrier of such molecules to type I diabetes and

celiac disease. Such HLA-DQ molecules are the combined protein products of specific HLA-DQB1 and DQA1 alleles known as DQB1*0201, DQB1*0302, DQB1*0304, DQB1*0401, DQB1*0501, DQB1*0502; and DQA1*0301, DQA1*0302, DQA1*0303, DQA1*0501. These alleles may be encoded on the same haplotypes ("cis" alleles) such as DQB1*0201-DQA1*0501-DRB1*0301 and DQB1*0302-DQA1*0301-DRB1*0401. The resulting HLA molecule comprising polypeptide products of "cis" alleles are herein referred to as "cis dimer." Alternatively, the alleles may be encoded on different haplotypes ("trans" alleles). The HLA molecule comprising polypeptide products of "trans" alleles are herein referred to as "trans" dimer. An example of "trans" alleles is the combination of DQB1*0201 on DQB1*0201-DQA1*0501-DRB1*0301 and DQA1*0301 on DQB1*0301-DQA1*0301-DRB1*0404.

[0015] In certain embodiments, the subject DQ-directed copolymers are a mixture of randomized or partially randomized amino acid sequence containing amino acids from each of the following four groups: (1) hydrophobic, aliphatic amino acids (such as leucine, isoleucine, valine, methionine); (2) amino acids with acidic side chains (such as aspartic acid, glutamic acid); (3) amino acids with small hydrophilic side chains (such as serine, cysteine, threonine); and (4) amino acids with small aliphatic side chains (such as alanine, glycine); additionally, the copolymer contains proline residues. In one embodiment, the copolymer is derived using the amino acids Glutamine (E) and/or Aspartic acid (D), Leucine (L), Serine (S) and Alanine (A), and is referred to herein as an "ELSA" copolymer.

[0016] In certain other embodiments, the subject DQ-directed copolymers are a mixture of randomized or partially randomized amino acid sequence containing amino acids from each of the following four groups: (1) hydrophobic, aliphatic amino acids (such as leucine, isoleucine, valine, methionine); (2) bulky hydrophobic amino acids (such as tyrosine, phenylalanine, leucine, methionine); (2) amino acids with acidic side chains (such as aspartic acid, glutamic acid); (3) amino acids with small hydrophilic side chains (such as serine, cysteine, threonine); and (4) amino acids with small aliphatic side chains (such as alanine, glycine); additionally, the copolymer contains proline residues. An exemplary copolymer is derived using the amino acid residues Glutamine (E) and/or Aspartic acid (D), Leucine (L), Tyrosine (Y) and Val (V), and is referred to herein as an "DLYV" copolymer.

[0017] In one embodiment, a method of treatment of an autoimmune disease comprises administration of a copolymer that binds to an HLA-DQ molecule associated with the

autoimmune disease. Preferably, the method of treatment is carried out using a copolymer that comprises a polypeptide comprising a plurality of amino acid residues selected from: (1) a hydrophobic, aliphatic residue (leucine, isoleucine, valine, methionine); (2) an acidic residue (aspartic acid, glutamic acid); (3) a small hydrophilic residue (serine, cysteine, threonine); (4) a small aliphatic residue (alanine, glycine); and (5) proline.

[0018] In preferred embodiments, the copolymers compositions of the present invention bind to one or more DQ isotypes with an average K_d of 1μM or less, and more preferably an average K_d less than 100nM, 10nM or even 1nM. Another way to identify preferred copolymers is based on competitive binding assays, such as described in Sidney *et al.* (2002) *J. Immunol.* 169:5098, which is expressed as an IC_{50} value. Preferred copolymers of the present invention have IC_{50}'s less than 1μM, more preferably less than 500nM, and even more less than 100nM.

[0019] In certain preferred embodiments, the copolymer is formed by random synthesis (polymerization) of the various amino acid residues. A certain ratio of amino acids to be incorporated into the random copolymer may be used. Preferred random copolymers of the present invention comprise amino acid residues K, E, A, S, V, and P. More preferably, the ratio of K:E:A:S:V is 0.3:0.7:9:0.5:0.5:0.3. Preferably, the random copolymers are about 10 to 100 amino acid residues long, more preferably 20 to 80 amino acid residues long, even more preferably 40 to 60 amino acid residues long, and most preferably about 50 amino acid residues long. When synthesized, a typical preparation of random copolymers is a mixture of peptides of various lengths, the majority of which are of the desired length but containing shorter or longer peptides inevitably created by the currently available synthetic processes.

[0020] In other embodiments, the copolymer has "anchor" residues which occur with regular spacing in the resulting polymer. Preferably, the copolymer can be synthesized to have one of the general sequences:

[0021] 1. $[XXEXXXXXXXEXX]_4$

[0022] 2. $[XXEXXXXXXXDXX]_4$

[0023] 3. $[XXDXXXXXXXDXX]_4$

[0024] 4. $[XXDXXXXXXXEXX]_4$

[0025] 5. $[XXEXXVXXXXDXX]_4$

[0026] 6. $[XXDXXVXXXXDXX]_4$

[0027] 7. $[XXDXXVXXXXEXX]_4$

[0028] 8. $[XXEXXVXXXXEXX]_4$

wherein X is A, S, V, K, or P.

[0029] In a preferred embodiment, the ratio of A:S:V:K:P is 5:1:1:1:0.5.

[0030] In certain preferred embodiments, the subject copolymers are formulated for use as a medicament so as to have a polydispersity less than 25,000, and more preferably less than 10000, 5000 or even 1000.

[0031] In certain embodiments, the subject copolymers bind to autoimmune-associated HLA-DQ istotypes, such as one or more of DQB1*0201, DQB1*0302, DQB1*0304, DQB1*0401, DQB1*0501, DQB1*0502; and DQA1*0301, DQA1*0302, DQA1*0303, DQA1*0501, with a K_d at least 10 times less than the copolymer's K_d for binding HLA-DR molecules and/or other DQ isotypes.

[0032] Another aspect of the present invention provides a pharmaceutical composition comprising a copolymer of the present invention. In certain embodiments, the pharmaceutical composition comprises one or more therapeutically effective copolymers that bind to HLA-DQ molecules, and a pharmaceutically acceptable carrier. More preferably, the therapeutically effective copolymers bind to HLA-DQ molecules associated with autoimmune diseases, thereby preventing induction of autoimmune responses. The pharmaceutical composition may be formulated for various routes of administration, including oral, intravenous, intramuscular, subcutaneous, transdermal, pulmonary or intraperitoneal administration. In another embodiment, the pharmaceutical composition is suitable for sustained release of the active ingredients, the composition comprising biologically compatible polymers or matrices that allow slow release of the therapeutically active copolymers.

[0033] In certain embodiments, the methods allow continuous treatment of autoimmune diseases by a sustained-release carrier such as transdermal patches, implantable medical devices

coated with sustained-release formulations, or implantable or injectable pharmaceutical formulation suitable for sustained-release of the active components.

[0034] The present invention also provides methods to prophylactically treat subjects that are at risk of developing autoimmune diseases so as to prevent or delay the onset of such diseases, comprising administering the copolymer.

[0035] In certain embodiments, the pharmaceutical composition further comprises other copolymers, such as copolymers that causes HLA-DR mediated activation of T cells. Exemplary DR-directed copolymers include Copaxone (glatiramer acetate, such as described in US Patents 3849550 and 6214791), YFAK and other copolymers described in PCT publication WO03/029276, and terpolymers described in PCT publication WO00/05250.

[0036] The subject copolymers can be co-formulated with or administered conjointly with other active ingredients, such as immunosuppressant agents and anti-inflammatory agents. For example, the subject copolymers can be used in conjunction with cyclooxygenase inhibitors, and inhibitors of TNF-α, IL-1 or ICAM-1.

[0037] In the case of treating IDDM, the copolymers of the present invention may also be co-formulated or conjointly administered with other known therapies for the treatment of diabetes, including PPAR agonists, sulfonylurea drugs, non-sulfonylurea secretagogues, α-glucosidase inhibitors, insulin sensitizers, insulin secretagogues, hepatic glucose output lowering compounds, and insulin. Such therapies may be administered prior to, concurrently with or following administration of the compound of the invention. Insulin includes both long and short acting forms and formulations of insulin. PPAR agonist may include agonists of any of the PPAR subunits or combinations thereof. For example, PPAR agonist may include agonists of PPAR-α, PPAR-γ, PPAR-67 or any combination of two or three of the subunits of PPAR. PPAR agonists include, for example, rosiglitazone and pioglitazone. Sulfonylurea drugs include, for example, glyburide, glimepiride, chlorpropamide, and glipizide. α-glucosidase inhibitors that may be useful in treating diabetes when administered with a copolymer of the invention include acarbose, miglitol and voglibose. Insulin sensitizers that may be useful in treating diabetes when administered with a subject copolymer include thiozolidinediones and non-thiozolidinediones. Hepatic glucose output lowering compounds that may be useful include metformin, such as Glucophage and Glucophage XR. Insulin secretagogues that may be useful in treating diabetes

when administered with a copolymer of the invention include sulfonylurea and non-sulfonylurea drugs: GLP-1, GIP, PAC/VPAC receptor agonists, secretin, nateglinide, meglitinide, repaglinide, glibenclamide, glimepiride, chlorpropamide, glipizide. GLP-1 includes derivatives of GLP-1 with longer half-lives than native GLP-1, such as, for example, fatty-acid derivatized GLP-1 and exendin.

[0038] Copolymers of the invention may also be used in combination with anti-obesity drugs. Anti-obesity drugs include P-3 agonists, CB-1 antagonists, appetite suppressants, such as, for example, sibutramine (Meridia), and lipase inhibitors, such as, for example, orlistat (Xenical).

[0039] The subject copolymers may also be used in methods of the invention in combination with drugs commonly used to treat lipid disorders in diabetic patients. Such drugs include, but are not limited to, HMG-CoA reductase inhibitors, nicotinic acid, bile acid sequestrants, and fibric acid derivatives. Polypeptides of the invention may also be used in combination with anti-hypertensive drugs, such as, for example, β-blockers, cathepsin S inhibitors and ACE inhibitors.

[0040] Another aspect of the present invention provides methods to screen for and identify copolymers that bind to HLA-DQ molecules and prevent autoimmune responses. Such methods allow to identify copolymers that are effective for treating autoimmune diseases.

[0041] In certain embodiments, the subject DQ-directed copolymers are modified, or labeled, with a moiety that facilitates the detection of the copolymers. In a preferred embodiment, the copolymers are biotinylated. In another preferred embodiment, the copolymers are modified with FITC. Exemplary copolymers are random copolymers as described above, modified with biotin or FITC. In other embodiments, the copolymers with "anchor" residues which occur with regular spacing in the resulting polymer are modified with biotin or FITC. Preferably, modified copolymers can be synthesized to have one of the general formulae:

[0042] 9. Biotin-spacer-XXEXXXXXXXEXX

[0043] 10. Biotin-spacer-XXEXXXXXXXDXX

[0044] 11. Biotin-spacer-XXDXXXXXXXDXX

[0045] 12. Biotin-spacer-XXDXXXXXXXEXX

[0046] 13. Biotin-spacer-XXEXXVXXXXDXX

[0047] 14. Biotin-spacer-XXDXXVXXXXDXX

[0048] 15. Biotin-spacer-XXDXXVXXXXEXX

[0049] 16. Biotin-spacer-XXEXXVXXXXEXX

wherein A, S, V, K, or P, the ratio of which are 5:1:1:1:0.5, and the spacer comprises two to 6 amino acid residues, preferably with the amino acid sequence SGSG.

[0050] These modified copolymers are used in assays and diagnostics, for example in enzyme-linked immunosorbent assay (ELISA). The labeled copolymers can also be used to determine the best sequence or preferred sequence among the copolymers that bind to an HLA molecule. Additionally, the labeled copolymer can be used in screening for other compounds not related to copolymers of the present invention but binds or associate with HLA-DQ molecules.

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

[0051] There are several autoimmune diseases that exhibit strong associations with certain alleles of human leukocyte antigen (HLA). In particular, certain diseases are associated with the HLA-DQ subclass of the alleles, either alone or in combination with the HLA-DR subclass. These diseases include type I diabetes mellitus and celiac disease. It is possible to identify individuals at risk of developing the diseases based on the identification of MHC class II alleles that confer susceptibility.

[0052] Type I diabetes is a serious health problem. Genetically susceptible individuals who possess certain HLA-DR and HLA-DQ subclass alleles may be monitored for autoantibodies to islet antigens which indicate onset of the disease. Treatment of such individuals at the onset of the disease to suppress autoimmune response and therefore any further destruction of the tissue is expected to be efficacious.

[0053] Another potential disease application is treatment of celiac disease, which is strongly associated with HLA encoded by alleles DQA1*0501-DQB1*0201 and -DQA1*03-DQB1*0302. Suppression of autoimmune response is expected to be efficacious in alleviating the symptoms of celiac disease.

[0054] T lymphocytes are able to recognize foreign antigen via their T cell receptor (TCR). The TCR binds to a major histocompatibility protein (MHC), which is a membrane bound glycoprotein on the cell surface of specialized antigen presenting cells. A MHC forms a complex with short, intracellularly processed peptides derived from self or foreign proteins. There are two major classes of MHC proteins, class I and class II. Class I molecules are complexed with such processed peptides derived from self or foreign proteins inside the cell, while class II molecules are in complex with those from outside the cell. Such peptide binds non-covalently to a MHC at its peptide binding groove, with a binding affinity (K_d) in the range of 10^{-6} M. The peptide binding groove of a class II MHC is open on either end and thus able to accommodate peptides of lengths ranging from 9 to 75 amino acid residues.

[0055] Peptide binding to class II molecules requires the presence of defined side chains at anchor positions, which all together form a particular binding motif. These anchor positions have been determined as amino acid positions 1-9 or P1 to P9. The most important contacts for a peptide to make for optimal class II binding are P1, P4, P7 and P9. Peptides with amino acid residues having distinguishing features of residues at specific positions bind to MHC molecules with predictable affinity. However, experiments using HLA-DR molecules show that, at non-anchor positions, a variation of side chains is permitted without influence on binding (Hammer *et al.*, (1993, 1994, and 1995), above). This binding mechanism enables the presentation of many different peptides by a given allotype of HLA. The side chains at anchor positions interact with specific pockets within the binding site, whereas those at non-anchor positions point outward, and are available for recognition by T cell receptors (TCR) on Th cells.

[0056] It is therefore conceivable that a compound with the same binding motif as autoantigenic peptides but with different residues at non-anchor positions would bind to the disease associated MHC molecules, thereby preventing the activation of autoimmune T cells, and thus interrupting the disease process. The mechanism whereby such a compound would exert its effect is competitive antagonism for the antigen-presenting site. Compounds binding selectively to class II molecules involved in a particular autoimmune disease are therefore

expected to interfere specifically with that disease. Additional peptides which bind to MHC molecules and inhibit T cell activation have been disclosed in, for example, International Patent applications WO 92/02543, WO 93/05011, WO 95/07707.

[0057] Alternatively, a compound that replaces an autoantigenic peptide may activate a different set of T cells than the autoantigenic peptide would. In instances where the autoimmune response is characterized by undesirable inflammatory responses mediated by Th1 cells, activation of Th2 cells instead of Th1 cells may alleviate the symptoms of the autoimmune reaction and result in suppression of undesired immune response.

[0058] Glatiramer acetate, also known as Copaxone or copolymer-1, is a random amino acid copolymer composed of tyrosine, lysine, glutamic acid and alanine. It has been successfully developed as a treatment for multiple sclerosis (MS). Although there is not a complete understanding of the mechanism of action of glatiramer acetate, it is likely that a pre-requisite for its biological activity involves an ability to bind human MHC class II molecules. The MHC allele most commonly associated with MS is HLA-DR2 (DRB1*1501), and glatiramer acetate has been shown to bind to this MHC class II molecule and activate a significant proportion (typically 15 – 20 %) of an individual's T cells. Activation of T cells by glatiramer acetate is restricted to HLA-DR molecules, and little response is generated through the HLA-DQ molecules. (Brenner *et al.* (2001) *J. Neuroimmunol.* 115:152-160, Fridkis-Hareli *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:4812-4876, Table 1).

Recently the crystal structure of a complex of a human insulin peptide and HLA encoded by alleles DQA1*03-DQB1*0302 has been determined. (Lee, K.H. *et al.* (2001) *Nature Immunol.* 2:501-507) Based on this structure and peptide binding studies with HLA encoded by alleles DQA1*03-DQB1*0302 (Yu, L. *et al.* (2000) *Eur. J. Immunol.* 30: 2497-2506), we predict that a copolymer containing amino acids from each of the following four groups would be able to bind to HLA-DQ molecules. Such groups are: (1) hydrophobic, aliphatic (leucine, isoleucine, valine, methionine); (2) acidic (aspartic acid, glutamic acid); (3) small hydrophilic (serine, cysteine, threonine); and (4) small aliphatic (alanine, glycine). Additionally, such copolymer contains proline residues. The acidic amino acid side chain serves as a key anchor residue for the P9 pocket of HLA encoded by alleles DQA1*03-DQB1*0302 and HLA encoded by alleles DQA1*0501-DQB1*0201, based on the β57 polymorphism that is linked to disease susceptibility. The aliphatic side chain serves as a good anchor for the second relevant pocket,

P4. The remaining pockets are best suited to accommodate small, neutral or hydrophobic residues. Therefore, in one embodiment, a copolymer that binds to an HLA-DQ molecule comprises a plurality of amino acid residues selected from the above-described four groups. In another embodiment, a copolymer that binds to an HLA-DQ molecule associated with the autoimmune disease comprises a polypeptide comprising a plurality of amino acid residues selected from: (1) a hydrophobic, aliphatic residue (leucine, isoleucine, valine, methionine); (2) an acidic residue (aspartic acid, glutamic acid); (3) a small hydrophilic residue (serine, cysteine, threonine); (4) a small aliphatic residue (alanine, glycine); and (5) proline. Such copolymers are at least 10 amino acid residues long, and may be 20, 30, 50, or even 100 amino acid residues long.

[0059] Further, in certain embodiments, the copolymer can be a semi-random (or semi-regular) polymer having "anchor," or fixed, residues which occur with regular spacing in the resulting polymer, providing for optimal class II binding. The anchor residues within the peptide may be E, D, or V. For example, the copolymer can be synthesized to have one of the general sequences:

[0060] 1. $[XXEXXXXXXXEXX]_n$

[0061] 2. $[XXEXXXXXXXDXX]_n$

[0062] 3. $[XXDXXXXXXXDXX]_n$

[0063] 4. $[XXDXXXXXXXEXX]_n$

[0064] 5. $[XXEXXVXXXXDXX]_n$

[0065] 6. $[XXDXXVXXXXDXX]_n$

[0066] 7. $[XXDXXVXXXXEXX]_n$

[0067] 8. $[XXEXXVXXXXEXX]_n$

wherein X is A, S, V, K, or P, the ratio of which are 5:1:1:1:0.5, and $1 \leq n \leq 8$.

[0068] The peptides may have a length of 9 to 25 amino acid residues. Preferably, the peptide is 13 amino acid residues long. A peptide of a defined sequence length of 9 to 25 amino

acids may contain from 2 to 20 fixed residues. An individual fixed residue of a peptide described in this invention may bind to the peptide binding grove of a class II MCH molecule at any of the positions P1, P4, P7, or P9. Preferably, such peptide contains 2 or 3 fixed residues. In one embodiment, a peptide of a defined sequence length of 13 amino acids will contain 2 fixed residues, either E or D or any combination thereof. Preferably a peptide of a defined sequence length of 13 amino acids will contain 3 fixed residues. The peptides may be multimers of a defined sequence, wherein the number of the repeating units preferably ranges from 2 to 8. More preferably, the number of the repeating units is 3 to 6. Most preferably, the number of repeating units is 4. In a preferred embodiment, a multimer of the instant invention comprises a peptide of a defined sequence length of 13 amino acids containing 2 fixed residues, either E or D or any combination thereof.

[0069] The present invention provides compounds that bind to and activate T cells in an HLA-DQ-mediated manner in addition to, or instead of, an HLA-DR-mediated manner. The present invention also provides compounds that bind to class II MHC molecules and prevent auto-antigenic peptides from activating T cells in an HLA-DQ-mediated manner.

[0070] The compounds of the invention which reduce HLA-DQ mediated autoimmune responses have therapeutic value in the prevention or treatment of various class II MHC-related diseases or disorders such as Insulin-dependent diabetes mellitus (IDDM), celiac disease, dermatitis herpetiformis and autoimmune thyroid disease (AITD). The compounds of the invention may be administered to a patient for treatment of an immune disorder, for example, involving undesirable or inappropriate immune activity, or may be used to prepare a therapeutic medicament. In particular, an effective dose of a compound of the invention may be therapeutically applied to ameliorate or to prevent insulin-dependent diabetes, celiac disease, and other diseases. An effective dose of a compound of the invention for the treatment of a disorder involving undesirable or inappropriate MHC activity, such as an autoimmune disorder, can be determined by standard means known in the art taking into account routine safety studies, toxicity studies, dose concentration studies and method of delivery, *e.g.*, bolus, continuous or repeated.

II. Definitions

[0071] The term "allotype" means a distinct antigenic form of a serum protein that results from allelic variations present on the immunoglobulin heavy chain constant region.

[0072] The term "associated with" means "coexistent with" or "in correlation with." The term does not necessarily indicate causal relationship, though such relationship may exist.

[0073] The term "binding" refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, ionic and/or hydrogen-bond interactions under physiological conditions, and including interactions such as salt bridges and water bridges.

[0074] The term "cis" refers to two alleles encoded by gene loci on the same haplotype while "trans" refers to two alleles encoded by genes on two different haplotypes. When two polypeptides that form an HLA protein are from cis alleles, the product is herein referred to as "cis dimer." When two polypeptides that form an HLA protein are from trans alleles, the product is herein referred to as "trans dimer."

[0075] The term "copolymer" means a polypeptide comprising plurality of amino acid residues of different kinds. Amino acid residues may be naturally occurring or synthetic analogs. Copolymers also include derivatives of a polypeptide, including chemically modified polypeptides and peptidomimetics.

[0076] The term "haplotype" is defined as a contiguous region of genomic DNA resulting from a non-random distribution of alleles on several gene loci of a same chromosome due to a low inter-chromosomal recombination in this particular region of the genome. As the MHC genes are proximal to each other on the chromosome, genetic recombination rarely occurs within the MHC and most individuals will inherit an intact set of parental alleles from each parent; such a set of linked genes is referred to as a haplotype, the MHC genes found in one haploid genome.

[0077] The term "HLA molecule" means any class II major histocompatibility complex glycoproteins. The term "HLA-DQ molecule" or "HLA-DR molecule" each refers to any one of HLA-DQ subtypes or HLA-DR subtypes.

[0078] The term "antigen binding groove" refers to a three dimensional antigen interactive site on the surface of the Class II MHC protein molecule (Stern, L.J. *et al.*, *Nature* 368:215 (1994)) that is formed by surfaces of both the α and β subunits of the Class II MHC protein molecule.

[0079] The term "MHC activity" refers to the ability of an MHC molecule to stimulate an immune response, *e.g.*, by activating T cells. An inhibitor of MHC activity is capable of suppressing this activity, and thus inhibits the activation of T cells by MHC. In preferred embodiments, a subject inhibitor selectively inhibits activation by a particular class II MHC isotype or allotype. Such inhibitors may be capable of suppressing a particular undesirable MHC activity without interfering with all MHC activity in an organism, thereby selectively treating an unwanted immune response in an animal, such as a mammal, preferably a human, without compromising the animal's immune response in general.

[0080] The term "patient" refers to an animal, preferably a mammal, including humans as well as livestock and other veterinary subjects.

[0081] The terms "peptide", "polypeptide" and "protein" are used interchangeably herein. These terms refer to unmodified amino acid chains, and also include minor modifications, such as phosphorylations, glycosylations and lipid modifications. The terms "peptide" and "peptidomimetic" are not mutually exclusive and include substantial overlap.

[0082] A "peptidomimetic" includes any modified form of an amino acid chain, such as a phosphorylation, capping, fatty acid modification and including unnatural backbone and/or side chain structures. As described below, a peptidomimetic comprises the structural continuum between an amino acid chain and a non-peptide small molecule. Peptidomimetics generally retain a recognizable peptide-like polymer unit structure. Thus, a peptidomimetic may retain the function of binding to a HLA protein forming a complex which activates autoreactive T cells in a patient suffering from an autoimmune disease.

[0083] The term "amino acid residue" is known in the art. In general the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (see *Biochemistry* (1972) 11:1726-1732). In certain embodiments, the amino acids used in the application of this invention are those naturally occurring amino acids found in proteins, or the naturally occurring anabolic or catabolic products of such amino acids which contain amino and carboxyl groups. Particularly suitable amino acid side chains include side chains selected from those of the following amino acids: glycine, alanine, valine, cysteine, leucine, isoleucine, serine,

threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan.

[0084] The term "amino acid residue" further includes analogs, derivatives and congeners of any specific amino acid referred to herein, as well as C-terminal or N-terminal protected amino acid derivatives (e.g. modified with an N-terminal or C-terminal protecting group). For example, the present invention contemplates the use of amino acid analogs wherein a side chain is lengthened or shortened while still providing a carboxyl, amino or other reactive precursor functional group for cyclization, as well as amino acid analogs having variant side chains with appropriate functional groups). For instance, the subject compound can include an amino acid analog such as, for example, cyanoalanine, canavanine, djenkolic acid, norleucine, 3-phosphoserine, homoserine, dihydroxy-phenylalanine, 5-hydroxytryptophan, 1-methylhistidine, 3-methylhistidine, diaminopimelic acid, ornithine, or diaminobutyric acid. Other naturally occurring amino acid metabolites or precursors having side chains which are suitable herein will be recognized by those skilled in the art and are included in the scope of the present invention.

[0085] The term "hydrophobic" amino acid means aliphatic amino acids alanine (A, or ala), glycine (G, or gly), isoleucine (I, or ile), leucine (L, or leu), proline (P, or pro), and valine (V, or val), the terms in parentheses being the one letter and three letter standard code abbreviations for each amino acid, and aromatic amino acids tryptophan (W, or trp), phenylalanine (F, or phe), and tyrosine (Y, or tyr). These amino acids confer hydrophobicity as a function of the length of aliphatic and size of aromatic side chains, when found as residues within a protein.

[0086] The term "charged" amino acid means amino acids aspartic acid (D or asp), glutamic acid (E or glu), histidine (H or his), arginine (R or arg) and lysine (K or lys), which confer a positive (his, lys, and arg) or negative (asp, gly) charge at physiological values of pH in aqueous solutions on proteins containing these residues.

[0087] Most of the amino acids used in the copolymers of the present invention may exist in particular geometric or stereoisomeric forms. In preferred embodiments, the amino acids used to form the subject copolymers are (L)-isomers, although (D)-isomers may be included in the copolymers such as at non-anchor positions or in the case of peptidomimetic versions of the copolymers.

[0088] "Prevent", as used herein, means to delay or preclude the onset of, for example, one or more symptoms, of a disorder or condition.

[0089] The term "prodrug" is intended to encompass compounds that, under physiological conditions, are converted into the inhibitor agents of the present invention. A common method for making a prodrug is to select moieties which are hydrolyzed under physiological conditions to provide the desired biologically active drug. In other embodiments, the prodrug is converted by an enzymatic activity of the patient or alternatively of a target pathogen.

[0090] "Treat", as used herein, means at least lessening the severity or ameliorating the effects of, for example, one or more symptoms, of a disorder or condition.

[0091] The term "ED_{50}" means the dose of a drug that produces 50% of its maximum response or effect. Alternatively, it may refer to the dose that produces a pre-determined response in 50% of test subjects or preparations.

[0092] The term "LD_{50}" means the dose of a drug that is lethal in 50% of test subjects.

[0093] The term "therapeutic index" refers to the therapeutic index of a drug defined as LD_{50}/ED_{50}.

[0094] The terms "structure-activity relationship" or "SAR" refer to the way in which altering the molecular structure of drugs alters their interaction with a receptor, enzyme, etc.

[0095] The term "aliphatic" refers to a linear, branched, cyclic alkane, alkene, or alkyne. In certain embodiments, aliphatic groups in the present invention are linear or branched and have from 1 to about 20 carbon atoms.

[0096] The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has about 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and alternatively, about 20 or fewer carbon atoms. Likewise, cycloalkyls have from about 3 to about 10 carbon atoms in their ring structure, and alternatively about 5, 6 or 7 carbons in the ring structure.

[0097] Moreover, the term "alkyl" (or "lower alkyl") includes both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents may include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain may themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls may be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, $-CF_3$, -CN, and the like.

[0098] The term "heteroatom" refers to an atom of any element other than carbon or hydrogen. Illustrative heteroatoms include boron, nitrogen, oxygen, phosphorus, sulfur and selenium, and alternatively oxygen, nitrogen or sulfur.

[0099] The term "aryl" includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring may be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at

least one of the rings is aromatic, e.g., the other cyclic rings may be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

III. Exemplary embodiments

Compounds

[00100] The compounds of the present invention are random or semi-random copolymers of amino acids residues described above or analogs thereof (such as to form peptidomimetics).

[00101] To illustrate, a copolymer of the invention can be synthesized using Fmoc or t-boc initiating amino acid analogs, or the like, which are immobilized on a resin in an automated peptide synthesis apparatus for further polymerization (solid state synthesis). The amino acids are polymerized in molar ratios that can be adjusted to provide a copolymer with optimal binding characteristics.

[00102] Synthesis procedures can include providing a solution which is a mixture of the chosen amino acids in an activated form, for example, activated as an N-carboxy anhydride, in the appropriate molar ratios of each of the appropriately derivatized amino acid precursors (derivatized to protect certain functional groups, such as the F, amino group of L-lysine, for example the precursor F,N-trifluoroacetyl-L-lysine). Alternatively, the synthesis procedure can involve online mixing during the synthetic procedure of derivatized precursors of the selected amino acids in the preferred molar ratios.

[00103] Examples of such resin supports for peptide synthesis include a Merrifield resin, chloromethylated polystyrene with 1% DVB cross-links; an Fmoc amino acid Wang resin, 4-benzyloxybenzyl alcohol, the resins being pre-loaded with an amino acid (for example, Fmoc-D-trp(boc)-Wang resin). Resins are available in different mesh sizes, for example 100-200 mesh, and high loading or low loading densities of fractionalization of the initiating amino acid.

[00104] In certain embodiments, the compounds of the present invention include such linear copolymers that are further modified by substituting or appending different chemical moieties. In one embodiment, such modification is at a residue location and in an amount sufficient to inhibit proteolytic degradation of the copolymer in a subject. For example, the amino acid modification may be the presence in the sequence of at least one proline residue; the residue is present in at least one of carboxy- and amino termini; further, the proline can be

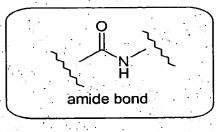
present within four residues of at least one of the carboxy- and amino-termini. Further, the amino acid modification may be the presence of a D-amino acid.

[00105] In certain embodiments, the subject copolymer is a peptidomimetic. Peptidomimetics are compounds based on, or derived from, peptides and proteins. The copolymer peptidomimetics of the present invention typically can be obtained by structural modification of one or more native amino acid residues, e.g., using unnatural amino acids, conformational restraints, isosteric replacement, and the like. The subject peptidomimetics constitute the continuum of structural space between peptides and non-peptide synthetic structures.

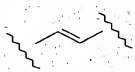
[00106] Such peptidomimetics can have such attributes as being non-hydrolyzable (e.g., increased stability against proteases or other physiological conditions which degrade the corresponding peptide copolymers), increased specificity and/or potency. For illustrative purposes, peptide analogs of the present invention can be generated using, for example, benzodiazepines (e.g., see Freidinger *et al.* in "Peptides: Chemistry and Biology," G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), substituted gamma lactam rings (Garvey *et al.* in "Peptides: Chemistry and Biology," G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988, p123), C-7 mimics (Huffman *et al.* in "Peptides: Chemistry and Biology," G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988, p. 105), keto-methylene pseudopeptides (Ewenson *et al.* (1986) *J. Med. Chem.* 29:295; and Ewenson *et al.* in "Peptides: Structure and Function (Proceedings of the 9th American Peptide Symposium)," Pierce Chemical Co. Rockland, IL, 1985), β-turn dipeptide cores (Nagai *et al.* (1985) *Tetrahedron Lett.* 26:647; and Sato *et al.* (1986) *J. Chem. Soc. Perkin Trans.* 1:1231), β-aminoalcohols (Gordon *et al.* (1985) *Biochem. Biophys. Res. Commun.*126:419; and Dann *et al.* (1986) *Biochem. Biophys. Res. Commun.* 134:71), diaminoketones (Natarajan *et al.* (1984) *Biochem. Biophys. Res. Commun.* 124:141), and methyleneamino-modifed (Roark *et al.* in "Peptides: Chemistry and Biology," G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988, p134). Also, see generally, Session III: Analytic and synthetic methods, in "Peptides: Chemistry and Biology," G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988)

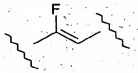
[00107] In addition to a variety of side chain replacements which can be carried out to generate the subject copolymer peptidomimetics, the present invention specifically contemplates the use of conformationally restrained mimics of peptide secondary structure. Numerous

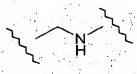
surrogates have been developed for the amide bond of peptides. Frequently exploited surrogates for the amide bond include the following groups (i) trans-olefins, (ii) fluoroalkene, (iii) methyleneamino, (iv) phosphonamides, and (v) sulfonamides.

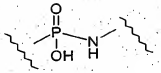


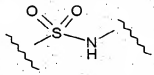
[00108] Examples of Surrogates



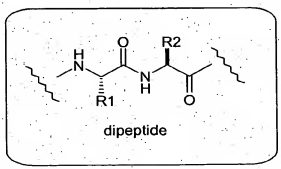




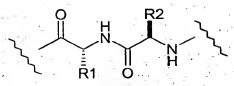




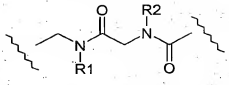
[00109] Additionally, peptidomimetics based on more substantial modifications of the backbone of the copolymer can be used. Peptidomimetics which fall in this category include (i) retro-inverso analogs, and (ii) N-alkyl glycine analogs (so-called peptoids).



[00110] Examples of analogs

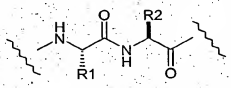


retro-inverso



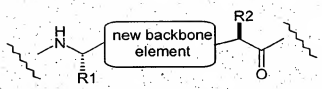
N-alkyl glycine

[00111] Furthermore, the methods of combinatorial chemistry are being brought to bear on the development of peptidomimetic copolymers. For example, one embodiment of a so-called "peptide morphing" strategy focuses on the random generation of a library of peptide analogs that comprise a wide range of peptide bond substitutes.



dipeptide

peptide morphing



[00112] In an exemplary embodiment, the peptidomimetic can be derived as a retro-inverso analog. Retro-inverso analogs can be made according to the methods known in the art, such as that described by the Sisto *et al.* U.S. Patent 4,522,752. As a general guide, sites which are most susceptible to proteolysis are typically altered, with less susceptible amide linkages being optional for mimetic switching The final product, or intermediates thereof, can be purified by HPLC.

[00113] In another illustrative embodiment, the peptidomimetic can be derived as a retro-enantio copolymer. Retro-enantio analogs such as this can be synthesized commercially

available D-amino acids (or analogs thereof) and standard solid- or solution-phase peptide-synthesis techniques.

[00114] In still another illustrative embodiment, trans-olefin derivatives can be made. A trans-olefin analog of a copolymer can be synthesized according to the method of Y.K. Shue *et al.* (1987) *Tetrahedron Lett.* 28:3225 and also according to other methods known in the art. It will be appreciated that variations in the cited procedure, or other procedures available, may be necessary according to the nature of the reagent used.

[00115] It is further possible to couple the pseudodipeptides synthesized by the above method to other pseudodipeptides, to make copolymers with several olefinic functionalities in place of amide functionalities. For example, pseudodipeptides corresponding to certain di-peptide sequences could be made and then coupled together by standard techniques to yield an analog of the copolymer peptide which has alternating olefinic bonds between residues.

[00116] Still another class of peptidomimetic derivatives includes phosphonate derivatives. The synthesis of such phosphonate derivatives can be adapted from known synthesis schemes. See, for example, Loots *et al.* in "Peptides: Chemistry and Biology," (Escom Science Publishers, Leiden, 1988, p. 118); Petrillo *et al.* in "Peptides: Structure and Function (Proceedings of the 9th American Peptide Symposium)," Pierce Chemical Co. Rockland, IL, 1985).

[00117] In other embodiments, the modification may be introduction of carbohydrate or lipid moieties. Such modifications also change the solubility of the copolymers into various medium so that they may advantageously be prepared into a suitable pharmaceutical composition. Modifying lipid groups include farnesyl group or myristoyl group. Modifying carbohydrate groups include single sugars or oligosaccharides of any naturally occurring and synthetic sugar and sugar alcohols, for example glucose, galactose, rhamnose, mannose, arabinose, and other sugars, and their respective alcohols.

[00118] The compounds of present invention comprise at least 15 amino acid residues, more preferably at least 20 amino acid residues, even more preferably at least 30 amino acid residues, most preferably at least 50 amino acid residues.

Therapeutic compositions

[00119] Another aspect of the present invention provides pharmaceutical composition comprising a pharmaceutically effective amount of copolymer of the present invention and an acceptable carrier and/or excipients. A pharmaceutically acceptable carrier includes any solvents, dispersion media, or coatings that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, oral, intraperitoneal, transdermal, topical, or subcutaneous administration. One exemplary pharmaceutically acceptable carrier is physiological saline. Other pharmaceutically acceptable carriers and their formulations are well-known and generally described in, for example, *Remington's Pharmaceutical Science* (18th Ed., ed. Gennaro, Mack Publishing Co., Easton, PA, 1990). Various pharmaceutically acceptable excipients are well-known in the art and can be found in, for example, *Handbook of Pharmaceutical Excipients* (4th ed., Ed. Rowe *et al.* Pharmaceutical Press, Washington, D.C.). The composition can be formulated as a solution, microemulsion, liposome, capsule, tablet, or other suitable forms. The active component which comprises the copolymer may be coated in a material to protect it from inactivation by the environment prior to reaching the target site of action.

[00120] In other embodiments of the present invention, the pharmaceutical compositions are sustained release formulations. Copolymers of the present invention may be admixed with biologically compatible polymers or matrices which control the release rate of the copolymers into the immediate environment. Controlled or sustained release compositions include formulation in lipophilic depots (*e.g.*, fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (*e.g.*, poloxamers or poloxamines). Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. Acceptable carriers include carboxymethyl cellulose (CMC) and modified CMC.

[00121] The pharmaceutical composition of the present invention is preferably sterile and non-pyrogenic at the time of delivery, and is preferably stable under the conditions of manufacture and storage.

[00122] The pharmaceutical composition may also include additional therapeutically active ingredients. Such additional ingredient can be another copolymer such as Copaxone that

binds to a different HLA molecule, an antibody which binds to an unwanted inflammatory molecule or cytokine such as interleukin-6, interleukin-8, granulocyte macrophage colony stimulating factor, and tumor necrosis factor-α; an enzyme inhibitor such as a protease inhibitor aprotinin or a cyclooxygenase inhibitor; an antibiotic such as amoxicillin, rifampicin, erythromycin; an antiviral agent such as acyclovir; a steroidal anti-inflammatory such as a glucocorticoid; a non-steroidal anti-inflammatory such as aspirin, ibuprofen, or acetaminophen; or a non-inflammatory cytokine such as interleukin-4 or interleukin-10. Other cytokines and growth factors such as interferon-β, tumor necrosis factors, antiangiogenic factors, erythropoietins, thrombopoietins, interleukins, maturation factors, chemotactic protein, and their variants and derivatives that retain similar physiological activities may also be used as an additional ingredient.

[00123] Copolymers of the invention may also be used in combination with anti-obesity drugs. Anti-obesity drugs include P-3 agonists, CB-1 antagonists, appetite suppressants, such as, for example, sibutramine (Meridia), and lipase inhibitors, such as, for example, orlistat (Xenical).

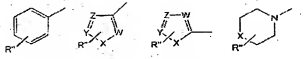
[00124] The subject copolymers may also be used in methods of the invention in combination with drugs commonly used to treat lipid disorders in diabetic patients. Such drugs include, but are not limited to, HMG-CoA reductase inhibitors, nicotinic acid, bile acid sequestrants, and fibric acid derivatives.

[00125] Polypeptides of the invention may also be used in combination with anti-hypertensive drugs, such as, for example, β-blockers, cathepsin S inhibitors and ACE inhibitors. Examples of β-blockers are: acebutolol, bisoprolol, esmolol, propanolol, atenolol, labetalol, carvedilol, and metoprolol. Examples of ACE inhibitors are: captopril, enalapril, lisinopril, benazepril, fosinopril, ramipril, quinapril, perindopril, trandolapril, and moexipril. Examples of cathepsin S specific inhibitors are: furanone derivatives having a structure represented by Formula (I) below:

(I)

wherein R1 = R', R'C(O), R'C(S), R'SO2, R'OC (O), R'NHC(O),

R' =



X = O, S, NH, W, Y, Z = CH, N;

R"= single or multiple ring substitution combinations taken from:

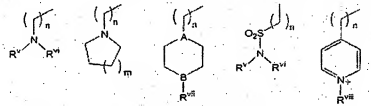
H, C1-7-alkyl, C3-6-cycloalkyl, OH, SH, Amine, Halogen;

R2, R4 = H, C1-7-alkyl, C3-7-cycloalkyl;

R3 = C1-7-alkyl, C3-7-cycloalkyl, Ar-Cl-7-alkyl;

R5 = C1-7-alkyl, halogen, Ar-C1-7-alkyl, Cl-3-alkyl-CONR'", R^{iv};

R^{iv} =



where n = 1-3, m = 1-3;

R^{v} , R^{vi} = H,C1-7-alkyl;

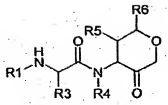
A = N, CH;

B = N, O, S, CH;

R^{vii} = absent when B = O, S; or R^{vii} = H, C1-7-alkyl when B = N, CH;

R^{viii}= O,C1-7-alkyl;

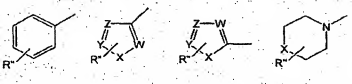
R6 = H, Ar-C1-7-alkyl, C1-3-alkyl-SO2-R^{ix}, C1-3-alkyl-C(O)-NHIR^{ix} or CH2XAr; where X and Ar are as defined herein; and pharmaceutically acceptable salts thereof. The compounds of formula (I) are disclosed in a published PCT application WO 00/69855, the disclosure of which is incorporated herein in its entirety. Other examples of cathepsin S inhibitors are furanone derivatives having a structure represented by Formula (II) below:



(II)

wherein R1 is R'-C(=O)- or R'-S(=O)2-

R' is



X=O, S, NH,

W,Y,Z = CH, N;

R^{v} = single or multiple ring substitution combinations taken from:

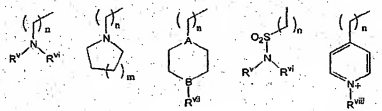
H, C1-7-alkyl, C3-6-cycloalkyl, OH, SH, amine, halogen;

R3 = C1-7-alkyl, C2-7-alkenyl, C3-7-cycloalkyl, Ar, Ar-C1-7-alkyl;

R4 = H, C1-7-alkyl, C3-7-cycloalkyl; C2-7-alkenyl, Ar, Ar-C1-7-alkyl;

R5 = C1-7-alkyl, hydroxyl- or halo-substituted C1-C7-alkylhalogen, Ar-C1-7-alkyl, C0-3-alkyl-CONR3R4 or R^{iv};

R^{iv} =



n = 1-3, m =1-3 ;

R^{v}, R^{vi} = H, C1-7-alkyl;

A = N, CH;

B=N, O, S, CH;

R^{vii} = absent when B = O, S; or R^{vii} = H, C1-7-alkyl when B = N, CH;

R^{viii} = O, C1-7-alkyl;

R6 = H, C1-7-alkyl, AR-C1-7-alkyl, C1-3-alkyl-SO2-R^{ix}, C1-3-alkyl-C(O)-NHR^{ix} or CH2XAr;

R^{ix} is C1-7-alkyl, Ar-C1-7-alkyl,C3-C6-cycloalkyl and pharmaceutically acceptable salts

thereof. The compounds of Formula (II) are disclosed in a published PCT application WO 02/40462, the disclosure of which is incorporated herein in its entirety. Examples of other cathepsin S inhibitors are: 1-[3-[4-(6-Chloro-2,3-dihydro-3-methyl-2-oxo-1H-benzimidazol-1-yl)-1-piperidinyl]propyl]-4,5,6,7-tetrahydro-5-(methylsulfonyl)-3-[4-(trifluoromethyl)phenyl]-1H-pyrazolo[4,3-c]pyridine (JNJ 10329670) (Thurmond *et al.* (2004) *J. Pharmacol. Exp. Ther.* 308(1):268-76, Epub 2003 Oct 17); CLIK-60 (Katunuma *et al. FEBS Lett.* 458:6-10); 4-morpholineurea-Leu-HomoPhe-vinylsulphone (Flannery *et al.* (2003) *Am. J. Pathol.* 163(1):175-82); Paecilopeptin (Shindo *et al.* (2002) *Biosci. Biotechnol. Biochem.* 66(11):2444-8); dipeptide nitriles (Ward *et al.* (2002) *J. Med. Chem.* 45(25):5471-82); dipeptide alpha-keto-beta-aldehydes (Walker *et al.* (2000) *Biochem. Biophys. Res. Commun.* 275(2):401-5).

Method of treatment

[00126] One aspect of the present invention provides for methods to treat a subject having an autoimmune disease by administering one or more copolymers of the present invention to the subject in a therapeutically effective amount.

[00127] In general, an embodiment of the invention is to administer a suitable daily dose of a therapeutic copolymer composition that will be the lowest effective dose to produce a therapeutic effect, for example, mitigating symptom. The therapeutic copolymers are preferably administered at a dose per subject per day of at least about 2 mg, at least about 5 mg, at least about 10 mg, or at least about 20 mg as appropriate minimal starting dosages. In one embodiment of the methods described herein, a dose of about 0.01 to about 500 mg/kg can be administered. In general, the effective dosage of the compound of the present invention is about 50 to about 400 micrograms of the compound per kilogram of the subject per day. However, it is understood by one skilled in the art that the dose of the composition of the invention will vary depending on the subject and upon the particular route of administration used. It is routine in the art to adjust the dosage to suit the individual subjects. Additionally, the effective amount may be based upon, among other things, the size of the compound, the biodegradability of the compound, the bioactivity of the compound and the bioavailability of the compound. If the compound does not degrade quickly, is bioavailable and highly active, a smaller amount will be required to be effective. The actual dosage suitable for a subject can easily be determined as a routine practice by one skilled in the art, for example a physician or a veterinarian given a general starting point.

[00128] The compound may be delivered hourly, daily, weekly, monthly, yearly (*e.g.*, in a time release form) or as a one-time delivery. The delivery may be continuous delivery for a period of time, *e.g.*, intravenous delivery. In one embodiment of the methods described herein, the agent is administered at least once per day. In one embodiment, the agent is administered daily. In one embodiment, the agent is administered every other day. In one embodiment, the agent is administered every 6 to 8 days. In one embodiment, the agent is administered weekly.

[00129] In one embodiment of the methods described herein, the route of administration can be oral, intraperitoneal, transdermal, subcutaneous, by intravenous or intramuscular injection, by inhalation, topical, intralesional, infusion; liposome-mediated delivery; topical, intrathecal, gingival pocket, rectal, intrabronchial, nasal, transmucosal, intestinal, ocular or otic delivery, or any other methods known in the art as one skilled in the art may easily perceive. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

[00130] An embodiment of the method of present invention is to administer the copolymers of the present invention in a sustained release form. Such method comprises applying a sustained-release transdermal patch or implanting a sustained-release capsule or a coated implantable medical device so that a therapeutically effective dose of the copolymer of the present invention is continuously delivered to a subject of such a method. The compounds and/or agents of the subject invention may be delivered via a capsule which allows sustained-release of the agent or the peptide over a period of time. Controlled or sustained-release compositions include formulation in lipophilic depots (*e.g.*, fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (*e.g.*, poloxamers or poloxamines). In certain embodiments, a source of a copolymer is stereotactically provided within or proximate to the area of autoimmune attack, for example, near the pancreas for the treatment of IDDM.

[00131] In another related embodiment, the methods further comprise administering at least one additional therapeutic agent. Such an agent may be another copolymer that binds to a second HLA molecule associated with the autoimmune disease, such as Copaxone; an antibody, an enzyme inhibitor, an antibacterial agent, an antiviral agent, a steroid, a nonsteroidal anti-inflammatory agent, an antimetabolite, a cytokine, or a soluble cytokine receptor. The second

HLA molecule may be an HLA-DQ molecule or an HLA-DR molecule. The enzyme inhibitor is a protease inhibitor or a cyclooxygenase inhibitor. The additional agent may be added as a part of the pharmaceutical composition, or may be administered concomitantly or within a time period when the physiological effect of the additional agent overlaps with the physiological effect of the copolymer of the present invention. More specifically, an additional agent may be administered concomitantly or one week, several days, 24 hours, 8 hours, or immediately before the administration of the copolymer. Alternatively, an additional agent may be administered one week, several days, 24 hours, 8 hours, or immediately after the administration of the copolymer.

[00132] The method of treatment provided by the present invention is suitable for treatment of Type I, or insulin-dependent, diabetes mellitus, celiac disease, or any other autoimmune disease mediated through HLA-DQ molecules.

[00133] Another embodiment of the present invention is a method for prophylactically treating a subject at risk of developing an autoimmune disease by administering a copolymer of the present invention. A subject at risk is identified by, for example, determining the genetic susceptibility to an autoimmune disease by testing for alleles of HLA that are associated with such autoimmune disease, and/or based on familial history, or other genetic markers that correlate with such autoimmune disease. Such prophylactic treatment may additionally comprise a second copolymer that binds to a second HLA molecule associated with the autoimmune disease to be treated. The second HLA molecule may be a HLA-DQ or HLA-DR molecule. Preferably, the autoimmune disease to be prophylactically treated is IDDM or celiac disease.

Method to identify therapeutically active copolymers

[00134] Another aspect of the present invention provides methods for identifying a therapeutic copolymer capable of reducing severity and frequency of episodes of an autoimmune disease.

[00135] In certain embodiments, the subject DQ-directed copolymers are modified, or labeled, with a moiety that facilitates the detection of the copolymers. In a preferred embodiment, the copolymers are biotinylated. In another preferred embodiment, the copolymers are modified with FITC. Exemplary copolymers are random copolymers as described above, modified with biotin or FITC. In other embodiments, the copolymers with "anchor" residues

which occur with regular spacing in the resulting polymer are modified with biotin or FITC. Preferably, modified copolymers can be synthesized to have one of the general formulae:

[00136] 9. Biotin-spacer-XXEXXXXXXXEXX

[00137] 10. Biotin-spacer-XXEXXXXXXXDXX

[00138] 11. Biotin-spacer-XXDXXXXXXXDXX

[00139] 12. Biotin-spacer-XXDXXXXXXXEXX

[00140] 13. Biotin-spacer-XXEXXVXXXXDXX

[00141] 14. Biotin-spacer-XXDXXVXXXXDXX

[00142] 15. Biotin-spacer-XXDXXVXXXXEXX

[00143] 16. Biotin-spacer-XXEXXVXXXXEXX

wherein A, S, V, K, or P, the ratio of which are 5:1:1:1:0.5, and the spacer comprises two to 6 amino acid residues, preferably with the amino acid sequence SGSG.

[00144] These modified copolymers are used in assays and diagnostics, for example in enzyme-linked immunosorbent assay (ELISA). The labeled copolymers can also be used to determine the best sequence or preferred sequence among the copolymers that bind to an HLA molecule. Additionally, the labeled copolymer can be used in screening for other compounds not related to copolymers of the present invention but binds or associate with HLA-DQ molecules.

[00145] A copolymer that is therapeutically effective to treat autoimmune disease can be identified by the following method: (1) a copolymer of the present invention is synthesized as described above; (2) determining binding of such copolymer to an HLA-DQ molecule; (3) comparing binding of the copolymer to the HLA-DQ molecule with binding of a known autoantigenic peptide to the HLA-DQ; (4) selecting a copolymer which binds to the HLA-DQ molecule substantially more strongly than the tested known autoantigenic peptide; and (5) determining activation of Th cells moderated by the HLA-DQ molecule presenting such selected copolymer.

[00146] Examples of an autoantigenic peptide are: a peptide comprising amino acid residues 9-23 of human insulin; a peptide comprising amino acid residues 206-220 of human GAD; or a peptide comprising amino acid residues 441-460 of human HSP60. The HLA-DQ molecule that the copolymer is tested against may be any HLA-DQ molecule described herein.

[00147] The ability of these new copolymers to bind to HLA-DQ molecules are tested by competitive binding assays in which the copolymer competes with a peptide derived from islet autoantigen, examples of which are listed above, for binding to soluble, recombinant HLA-DQA1*03-DQB1*0302. The soluble recombinant HLA-DQ8 encoded by alleles DQA1*03-DQB1*0302 was expressed in *Drosophila melanogaster* S2 cells under the control of the copper-inducible metallothionein promoter. HLA-DQ8 was engineered to be a soluble protein by replacing the transmembrane and intracellular segments of DQ α and DQ β with leucine zipper dimerization domains from the transcription factors Fos and Jun. See Hausmann, H.D. *et al.* (1999) *J. Exp. Med.* 189: 1723-1734. The expressed recombinant protein was purified from the concentrated supernatants by affinity chromatography using monoclonal antibody 9.3.F10 (HB 180, American Type Culture Collection) and anion-exchange chromatography using Mono Q HR column (Pharmacia Biotech).

[00148] Different copolymers were separated and pool sequenced as described in Stern, L.J. *et al.* (1994) *Nature* 368:215-221. Generally, fractionation of the polymers was by microbore HPLC using a Zorbax C18 1.0 mm reverse-phase column, eluted with a gradient of 0.055% trifluoroacetic acid in acetonitrile gradient of 0 to 60%. Peak selection, separation by reverse phase, and Edman microsequencing were devised based on Chicz, R.M. *et al.* (1993) *J. Exp. Med.* 178:27-47.

[00149] Copolymer binding assays were performed with biotinylated copolymers. In a binding assay, copolymer candidates and soluble HLA-DQ8 were incubated and the formed complex was captured using monoclonal antibody 9.3.F10, which binds specifically to HLA-DQ8. The captured complex was quantitated by detection with europium-labeled streptavidin. In a competition assay, biotinylated copolymers were preincubated with soluble HLA-DQ8. Unlabeled competitor peptides derived from islet autoantigens (such as insulin amino acid residues 9-23, GAD amino acid residues 206-220, or HSP60 amino acid residues 441-460) in excess amounts were then added to displace the copolymer. The fraction of copolymer-HLA-

DQ8 complexes remaining was measured up to 72 hours to determine the half-life of such complexes.

[00150] Copolymers with binding affinity stronger than or comparative to the autoantigenic peptides are selected. Preferred copolymers form complexes with HLA-DQ8 with a half-life of longer than 12 hours. More preferably, copolymers to be selected form complexes with HLA-DQ8 with a half-life of longer than 24 hours, 48 hours, or even more preferably, 72 hours.

[00151] To further test the ability of the copolymers selected by the binding assays to activate human T cells in a HLA-DQ restricted manner, the copolymers are incubated with human PBMCs from subjects with the HLA-DQ2 encoded by alleles DQA1*0501-DQB1*0201 or HLA-DQ8 encoded by alleles DQA1*03-DQB1*0302 allele. The restriction element(s) for the resulting cell lines can be determined with anti-DR and anti-DQ antibodies.

IV. Equivalents

[00152] Contemplated equivalents of the copolymers, subunits and other compositions described above include such materials which otherwise correspond thereto, and which have the same general properties thereof (e.g., biocompatible, antineoplastic), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of such molecule to achieve its intended purpose. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants that are in themselves known, but are not mentioned here.

[00153] All of the above-cited references are hereby incorporated by reference in their entireties.

CLAIMS

What is claimed is:

1. A method of treatment of an autoimmune disease comprising administration of a copolymer that binds to an HLA-DQ molecule associated with said autoimmune disease.

2. The method of claim 1, wherein said copolymer comprises a polypeptide comprising a plurality of amino acid residues selected from:

   (1) hydrophobic, aliphatic residues (leucine, isoleucine, valine, methionine);

   (2) acidic residues (aspartic acid, glutamic acid);

   (3) small hydrophilic residues (serine, cysteine, threonine);

   (4) small aliphatic residues (alanine, glycine); and

   (5) proline.

3. The method of claim 1, further comprising a second copolymer that binds to a second HLA molecule associated with said autoimmune disease.

4. The method of claim 3, wherein said second HLA molecule is an HLA-DQ molecule.

5. The method of claim 3, wherein said second HLA molecule is an HLA-DR molecule.

6. The method of any of claims 1 to 5, wherein said autoimmune disease is selected from type I diabetes mellitus and celiac disease.

7. A copolymer comprising plurality of amino acid residues selected from:

   (1) hydrophobic, aliphatic residues (leucine, isoleucine, valine, methionine);

   (2) acidic residues (aspartic acid, glutamic acid);

   (3) small hydrophilic residues (serine, cysteine, threonine);

   (4) small aliphatic residues (alanine, glycine); and

   (5) proline.

wherein the copolymer functionally binds to an HLA-DQ molecule.

8. The copolymer of claim 7, wherein the copolymer comprises at least 10 amino acid residues.

9. The copolymer of claim 8, wherein the copolymer comprises at least 20 amino acid residues.

10. The copolymer of claim 9, wherein the copolymer comprises at least 30 amino acid residues.

11. The copolymer of claim 10, wherein the copolymer comprises at least 50 amino acid residues.

12. The copolymer of claim 7, wherein the copolymer is further modified to facilitate detection.

13. The copolymer of claim 12, wherein the copolymer is modified by adduction of biotin or FITC.

14. A pharmaceutical composition for treatment of an autoimmune disease comprising a pharmaceutically effective amount of a copolymer that binds to an HLA-DQ molecule associated with an autoimmune disease.

15. The composition of claim14, wherein said copolymer is a copolymer of any of claims 7-11.

16. A method for prophylactically treating a subject at risk of developing an autoimmune disease, comprising administering a first copolymer that binds to a first HLA-DQ molecule associated with said autoimmune disease.

17. The method of claim 16, wherein said first copolymer comprises a polypeptide comprising plurality of amino acid residues selected from:

(1) a hydrophobic, aliphatic residue (leucine, isoleucine, valine, methionine);

(2) a acidic residue (aspartic acid, glutamic acid);

(3) a small hydrophilic residue (serine, cysteine, threonine);

(4) a small aliphatic residue (alanine, glycine); and

(5) proline.

18. The method of claim 16, further comprising a second copolymer that binds to a second HLA molecule associated with said autoimmune disease.

19. The method of claim 18, wherein said second HLA molecule is a HLA-DQ molecule.

20. The method of claim 18, wherein said second HLA molecule is a HLA-DR molecule.

21. The method of any of claims 16 to 20, wherein said autoimmune disease is selected from type I diabetes mellitus and celiac disease.

22. A method for identifying a copolymer that is therapeutically effective to treat an HLA-DQ mediated autoimmune disease comprising:

(a) synthesizing a random copolymer of amino acids selected from:

(1) hydrophobic, aliphatic residues (leucine, isoleucine, valine, methionine);

(2) acidic residues (aspartic acid, glutamic acid);

(3) small hydrophilic residues (serine, cysteine, threonine);

(4) small aliphatic residues (alanine, glycine); and

(5) proline.

(b) determining binding of said copolymer to an HLA-DQ molecule;

(c) comparing binding of said copolymer to said HLA-DQ molecule with binding of a known autoantigenic peptide to said HLA-DQ;

(d) selecting said copolymer which binds to said HLA-DQ molecule substantially more strongly than said known autoantigenic peptide; and

(e) determining activation of Th cells moderated by said HLA-DQ molecule presenting said copolymer.

23. The method of claim 22, wherein said autoantigenic peptide is selected from:

(1) a peptide comprising amino acid residues 9-23 of human insulin;

(2) a peptide comprising amino acid residues 206-220 of human GAD; and

(3) a peptide comprising amino acid residues 441-460 of human HSP60.

24. The method of claim 22, wherein said HLA-DQ molecule is DQA1*03-DQB1*0302DQA1*0501-DQB1*0201 a trans dimer between HLA-DQA1*0501-DQB1*0201 and HLA-DQA1*03-DQB1*0302, selected from DQA1*0301/B1*0302, DQB1*0201/DQA1*0501, DQB1 *0201 and DQA1*0301.

25. The method of any of claims 22 to 24, wherein the copolymer is biotinylated.

26. The method of any of claims 22 to 24, wherein the copolymer is labeled with FITC.

ABSTRACT

The present invention provides methods and compositions for treating autoimmune diseases and other unwanted immune reactions comprising administering a copolymer that binds to one or more HLA-DQ molecules and modulates DQ-restricted T cell responses. The copolymers are random copolymers of amino acids and copolymers comprising anchor residues to facilitate binding to the DQ binding pockets.